bio-protocol Preprint

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Culture conditions

Milo S Johnson Michael M Desai

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An abbreviated version of this protocol was published in eLIFE in Jan 2021

Phenotypic and molecular evolution across 10,000 generations in laboratory budding yeast populations

DOI: 10.7554/eLife.63910

Detailed protocol

Media and incubation conditions

(from Methods section): "We propagated all populations in 128 µL of media in unshaken flat-bottom polypropylene 96-well plates (VWR #82050–786). For one environment, we used rich YPD media (1% Bacto yeast extract (VWR #90000–726), 2% Bacto peptone (VWR #90000–368), 2% dextrose (VWR #90000–904)) and grew populations at 30°C. For the other two environments, we used synthetic complete (SC) media (0.671% YNB with nitrogen (Sunrise Science #1501–250), 0.2% SC (Sunrise Science # 1300–030), 2% dextrose) and grew populations at 30°C or 37°C. All media was supplemented with 100 µg/ml ampicillin and 25 µg/ml tetracycline."

Transfer protocol

Each day, we:

1. Remove plates from temperature-controlled incubators.
2. For each environment, fill a 96-well plate with 124 µl of media using the Biomek FXp liquid handling robots and one quadrant of a shared 384-well plate (VWR #82051-306) with 62 µl of media.
3. Resuspend cultures by shaking at 1200 rpm for 2 min on a Titramax 100 plate shaker (Heidolph Instruments).
4. For each environment, use the Biomek to perform the following actions:
    1. Pipet to mix the plate containing saturated culture (mix 25 µl 4 times).
    2. Aspirate 10 µl (YPD 30°C and SC 30°C) or 12 µl (SC 37°C) from the culture, immediately dispense 4 µl (used as a buffer volume to improve pipetting accuracy), dispense 2 µl (YPD 30°C and SC 30°C) or 4 µl (SC 37°C) into the appropriate quadrant of the 384-well plate, and dispense the remaining 4 µl back into the original plate with saturated culture (again, used as a buffer volume).
    3. Pipet to mix in the appropriate quadrant of the 384-well plate (mix 25 µl 6 times).
    4. Aspirate 12 µl (YPD 30°C and SC 30°C) or 16 µl (SC 37°C) from the appropriate quadrant of the 384-well plate, immediately dispense 4 µl (used as a buffer volume to improve pipetting accuracy), dispense 4 µl (YPD 30°C and SC 30°C) or 8 µl (SC 37°C) into the appropriate 96-well plate with fresh media, and dispense the remaining 4 µl back into the appropriate quadrant of the 384-well plate (again, used as a buffer volume).
    5. Wash the tips (VWR #89204–794) by pipet mixing 30 µl 5 times in water (to wash out cells), then in 100% ethanol (to lyse residual cells), and finally in air (to aid in drying), before returning the tips to their box. Each environment has a dedicated set of tips, which are left to dry in their box each night.
5. Shake the new 96-well plates at 1200 rpm for 1 min on a Titramax 100 plate shaker (Heidolph Instruments) to distribute cells evenly in each well.
6. Place these new plates in temperature-controlled incubators.

Plate reuse

The 96-well microplates used to maintain populations were bleached (to lyse cells), washed with distilled water, allowed to dry, and autoclaved (121°C, 30 min) before being reused.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Johnson, M. and Desai, M. (2022). Culture conditions. Bio-protocol Preprint. bio-protocol.org/prep1592.
2. Johnson, M. S., Gopalakrishnan, S., Goyal, J., Dillingham, M. E., Bakerlee, C. W., Humphrey, P. T., Jagdish, T., Jerison, E. R., Kosheleva, K., Lawrence, K. R., Min, J., Moulana, A., Phillips, A. M., Piper, J. C., Purkanti, R., Rego-Costa, A., McDonald, M. J., Nguyen Ba, A. N. and Desai, M. M.(2021). Phenotypic and molecular evolution across 10,000 generations in laboratory budding yeast populations. eLIFE. DOI: 10.7554/eLife.63910